REMARKS/ARGUMENTS

I. Status of the claims

Claim 107 is amended. Claims 107, 110, 111, 116-133 are pending, of which claims 110, 125-128, and 130-131 are withdrawn as directed to unelected species. Therefore, claims 107, 111, 116-124, 129, 132 and 133 are pending and currently under consideration.

II. Support for the amendments

The amendment to claim 107 merely restates the concluding step in the preamble. No new matter is added.

III. Elections/restrictions

The Examiner stated that the restriction was made final. Per page 3 of the Office Action, Applicants respectfully request that the Examiner re-combine and allow claims 110 and 123-131 should the Examiner find that claim 107 (designated by the Examiner as a "linking claim") is allowable.

IV. Rejection under 35 U.S.C. § 112, second paragraph

Claims 107, 116-124, 129, 132 and 133 were rejected as allegedly indefinite for not reciting a final process step that relates back to the preamble of the claim. In this context, the Examiner questioned whether the claim was directed to determination of methylation profiles or quantifying methylated or unmethylated sequences.

As amended, the preamble has been amended to recite that the claim is directed to "determining the relative amount of methylated or unmethylated DNA comprising a sequence." However, it should be noted that determining the relative amount of methylated or unmethylated DNA comprising a sequence allows one to generate a methylation profile for that particular sequence.

Accordingly, Applicants respectfully request withdrawal of the rejection.

V. Rejection under 35 U.S.C. § 103

Claims 107, 111, 116, 117, 119-124, 129, 132, and 133 were rejected as allegedly obvious over Huang *et al.* in view of Oefner *et al.* Specifically, the Examiner argued that Huang *et al.* described all of the limitations of claim 107 (*see*, Office Action, pages 5-7), except Huang *et al.* described use of a restriction enzyme to fragment DNA instead of the random fragmentation or shearing recited in the claim. The Examiner argued that it would have been obvious to use random fragmentation in view of Oefner *et al.*, which allegedly teaches use of random fragmentation to improve representation of underrepresented fragments, improved control over size of fragments, and size distribution independent of type of DNA used (*see*, Office Action, page 8).

Applicants respectfully traverse the rejection. Applicants submit that neither Huang *et al.* nor Oefner *et al.* describe "depleting methylated or unmethylated DNA from the second portion" as recited in step b of claim 107 and therefore all of the elements of the claims are not described in the cited art. Secondly, even if all of the elements are in fact in the cited art (which Applicants dispute), there was no motivation to combine the references as the Examiner suggested because the fragmentation by restriction enzyme as described by Huang *et al.* was performed for a specific purpose (retaining intact CpG islands) that would not have lead one of skill in the art to replace the restriction enzyme with random fragmentation or shearing. Accordingly, a *prima facie* rejection has not been set forth.

All claim elements are not described in the cited art

Step b of claim 107 is directed to "depleting methylated or unmethylated DNA from the second portion." "Depleting" as used in the specification, refers to *removal* of the relevant DNA sequences. For example, paragraph **[84]** of the specification defines "[a] sample 'depleted for methylated DNA'" as a sample "from which a majority of fragments containing methylated nucleotides at a sequence of interest ... have been *removed*" (italics added). Similarly, paragraph **[85]** of the specification defines "[a] sample 'depleted for unmethylated DNA'" as a sample "from which a majority of fragments containing unmethylated nucleotides at a sequence of interest ... have been *removed*" (italics added).

On page 6 of the Office Action, the Examiner argues that step b of claim 107 is described by Huang *et al.* because Huang *et al.* describes "depleting the unmethylated DNA from the second portion by digesting the DNA with BstU I enzyme which degrades unmethylated DNA (Fig. 2; page 468, second paragraph)." Huang *et al.* does not describe a method comprising *depletion* (i.e., removal) of unmethylated DNA as the Examiner suggests. Huang *et al.* describes digestion of the DNA with BstU I, which cleaves the unmethylated DNA, thereby generating shorter unmethylated DNA fragments, followed by PCR. While the treatment with BstU I renders the unmethylated DNA ineffective as a template for PCR, the treatment with BstU I does not deplete or remove the unmethylated DNA from the portion. Therefore, it is incorrect to characterize the Huang *et al* reference as describing step b of claim 107. In view of this omission, the rejection does not meet the requirements of a *prima facie* obviousness rejection. It should be noted that Oefner *et al.* does not address this aspect of the claimed method.

No motivation to combine the references

In addition, even if Huang *et al.* described step b of claim 107 (which Applicants dispute as described above), there was no motivation to combine the references as the Examiner has suggested. According to the Examiner, Huang *et al.* described all of the elements of claim 107 *except* use of random fragmentation or shearing as recited in step a of claim 107. The Examiner argued that it would have been obvious to use random fragmentation in view of Oefner *et al.*, which allegedly teaches use of random fragmentation to improve representation of underrepresented fragments, improved control over size of fragments, and size distribution independent of type of DNA used (*see*, Office Action, page 8). Thus, the Examiner argues it would have been obvious to replace the restriction enzyme (*Mse*I) used by Huang *et al.* in an initial fragmentation step (*see, e.g.*, Huang *et al.*, page 460, paragraph bridging columns 1 and 2 and Figure 2) with random fragmentation or shearing as described by Oefner *et al.*

Review of Huang *et al.*, page 460, paragraph bridging columns 1 and 2 reveals that *Mse*I was specifically selected as an endonuclease that cleaves at a recognition sequence that "rarely occurs within GC-rich regions, leaving most CpG islands intact." Further, Huang *et al.*

emphasizes the advantage of analyzing CpG islands on page 459, paragraph bridging columns 1 and 2:

> Most cytosines within CpG dinucleotides are methylated in the human genome, but some remain unmethylated in specific CpG-rich areas, called CpG islands [reference citation omitted]. These 1-2 kb long DNA sequences are located in the promoter and first exon regions of ~60% of all genes [reference citation omitted].

Further emphasis of CpG islands can be found in Huang *et al.*, among other places, in the title ("Methylation profiling of *CpG islands* in human breast cancer cells" (italics added)), as well as in column 2 of page 459 (describing, among other things, "[t]he molecular mechanisms underlying CpG island hypermethylation in cancer ...."). Indeed, this is consistent with the conclusion of the Huang *et al.* article, which states that one of the three unique features of the described method was "the genomic fragments were derived from a library specifically constructed to contain highly enriched CpG island sequences." *See*, Huang, *et al.*, page 464, column 2.

In view of the strong emphasis of Huang *et al.* for the study of CpG islands and the emphasis on the use of a restriction enzyme (*Mse*I) that allows for CpG islands to remain intact, Applicants submit that one of ordinary skill in the art would *not* have been lead to use an initial random fragmentation or shearing step instead of the *Mse*I digestion because of the emphasis in Huang *et al.* of the advantages of selecting an initial restriction enzyme that preserves CpG islands and therefore is *not* random. Thus, all of the "benefits" of random fragmentation allegedly described in Oefner *et al.* (e.g., obtaining "fragments with few restriction sites ... [in] underrepresented restriction digests" as cited in the Office Action, page 8), if considered at all, would have been considered *disadvantageous* in view of the emphasis of retaining intact CpG islands as set forth in Huang *et al.* Accordingly, there was no motivation in the art to combine Huang *et al.* and Oefner *et al.* as the Examiner has suggested.

Rejection in further view of Bestor *et al.*

The Examiner also rejected claim 118 as allegedly obvious over Huang *et al.* and Oefner *et al.* in further view of Bestor *et al.* As discussed above, the combination of Huang *et al* and Oefner *et al.* do not render claim 107 obvious. Claim 118 depends from claim 107 and therefore includes all of the limitations of claim 107. Bestor *et al.* does not cure the defects of Huang *et al.* and Oefner *et al.* with regard to either claim 107 or 118. Therefore, a *prima facie* rejection of claim 118 has not been set forth.

Request for withdrawal of the rejections

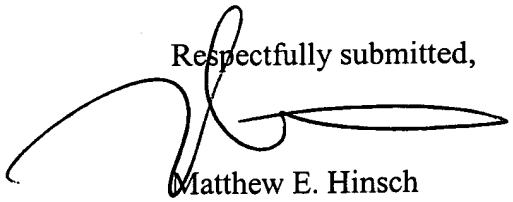
As the cited art did not describe all of the elements of the claims and there was no motivation to combine the references as suggested by the Examiner, Applicants respectfully request withdrawal of the obviousness rejections.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



Matthew E. Hinsch
Reg. No. 47,651

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300
Attachments
MEH:meh
60794627 v1